

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
SUBSTRATES FOR SYNTHESIS OF TRANSCRIPTION PRODUCTS
CORRESPONDING TO TARGET SEQUENCES

Inventor(s): Gary A. Dahl/Jerome J. Jendrisak/Elena K. Davydova/
Lucia B. Rothman-Denes/Svetlana Y. Gerdes

Application No.:

Docket Number: 310307.00003

1/25

Bacteriophage N4 vRNAP promoters

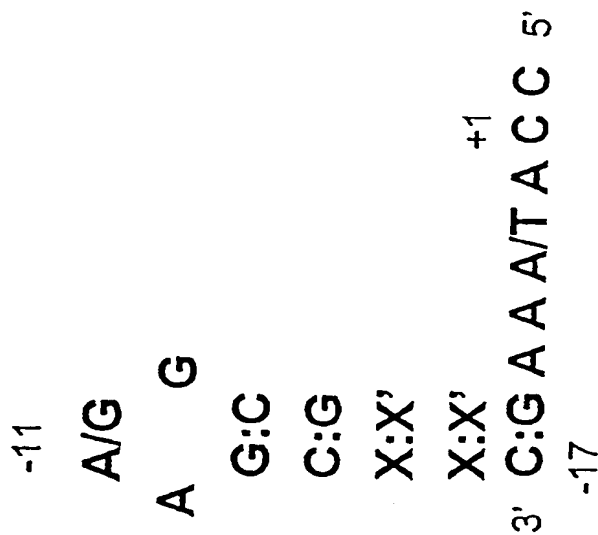


FIG. 1

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N4 vRNAP and generation of mini-vRNAP

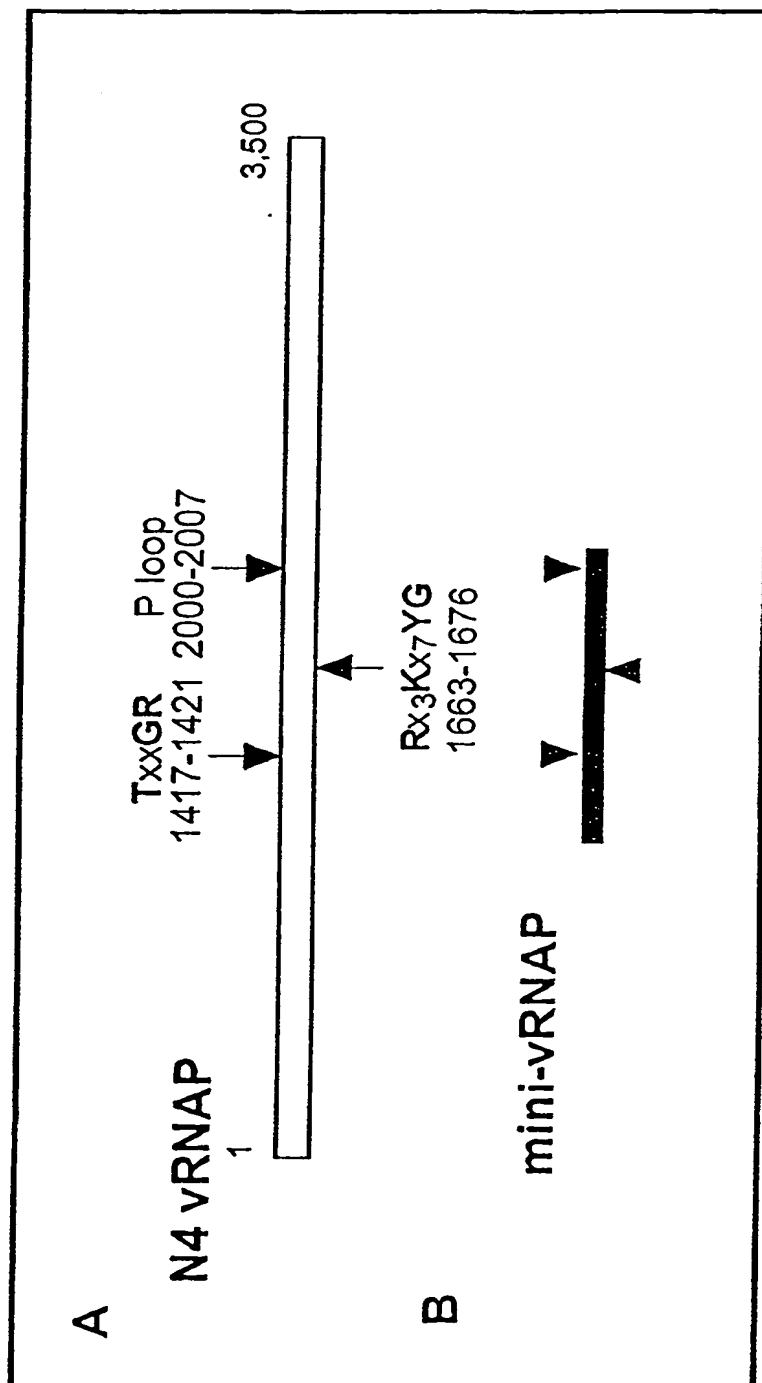


FIG. 2

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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Identification of the minimal active domain of N4 vRNAP
by proteolytic cleavage.

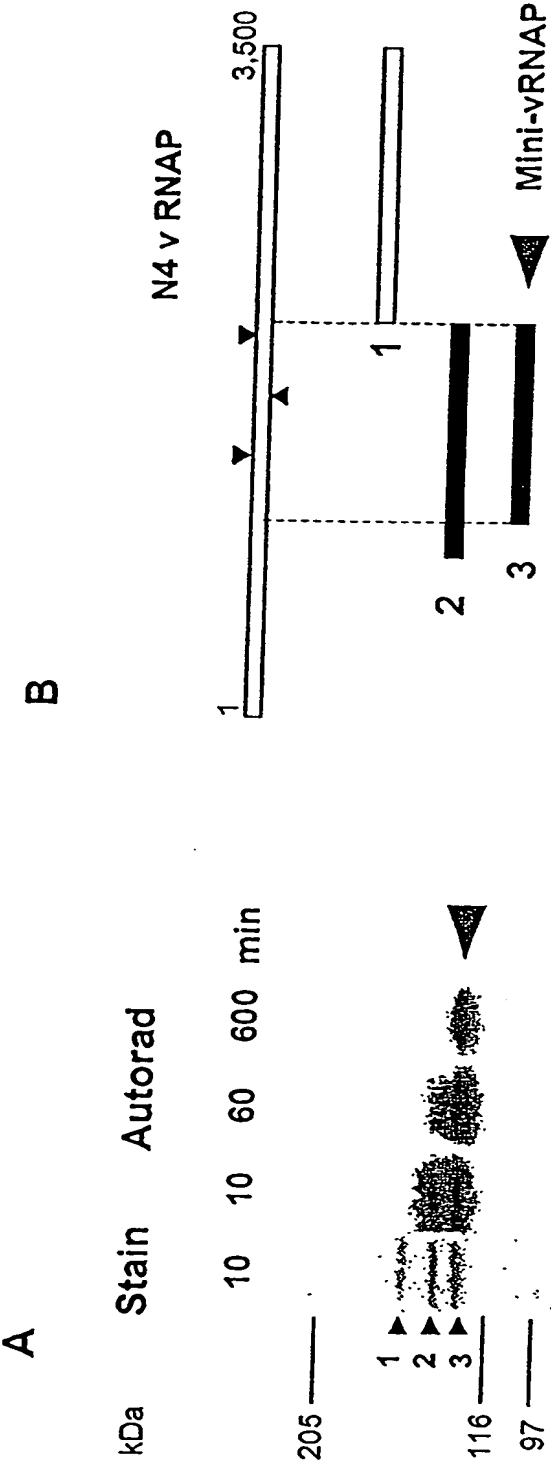


FIG. 3

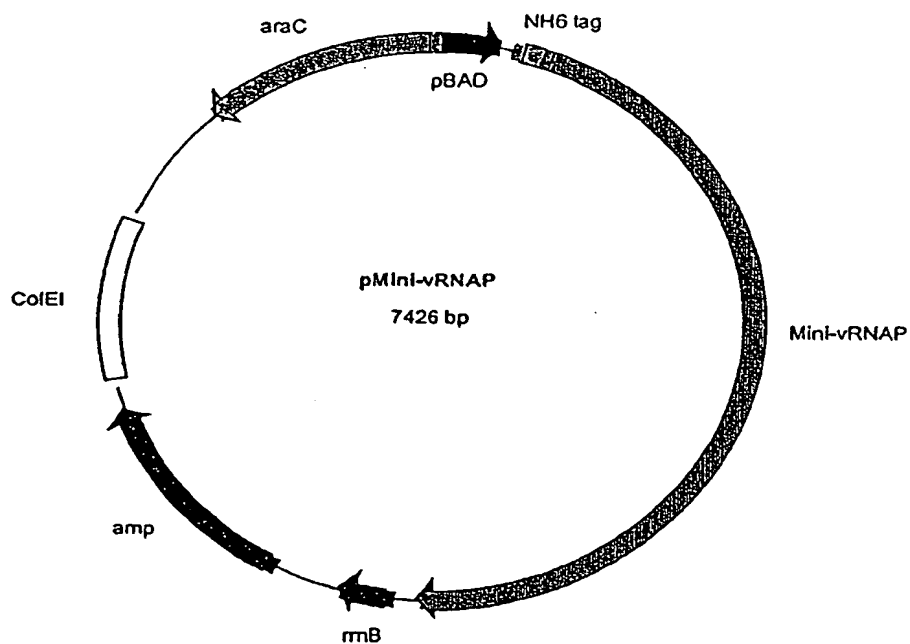
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Plasmid name: pMini-vRNAP
Plasmid size: 7426 bp
Constructed by: K. M. Kazmierczak
Construction date: 2/2000
Comments: Insert cloned into Invitrogen
pBAD 8 expression plasmid

FIG 4

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Purification of cloned vRNAP and mini-vRNAP

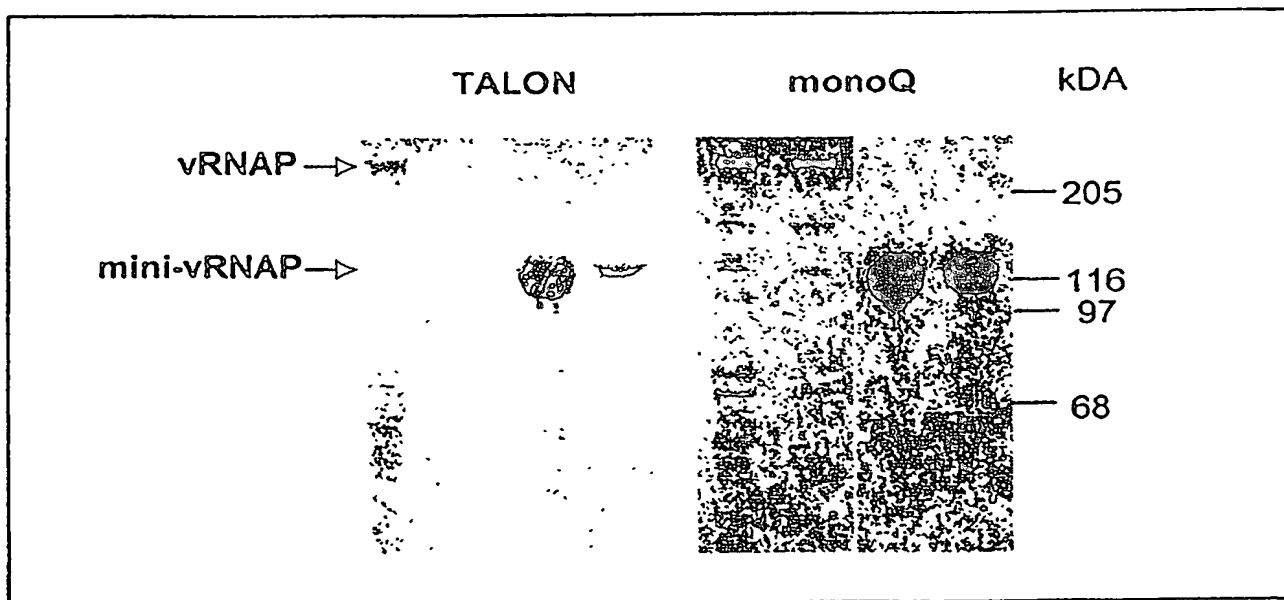


FIG 5

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Activation of N4 vRNAP transcription by *Eco* SSB
at different ssDNA concentrations

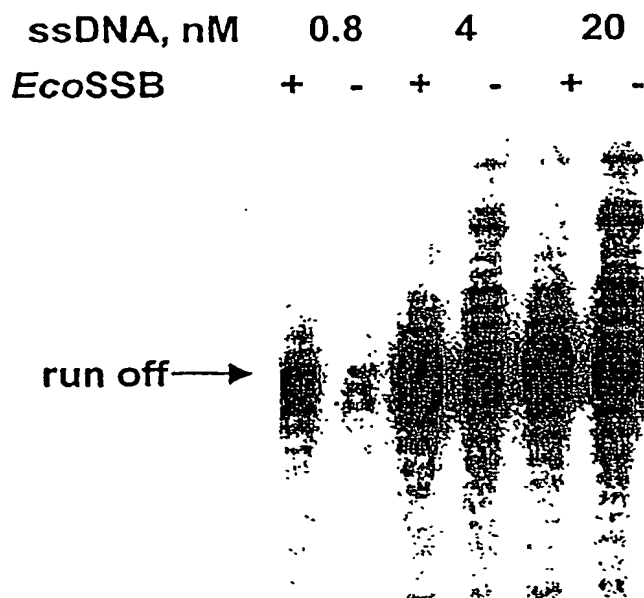


FIG 6

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Effect of Eco SSB on ssDNA template recycling


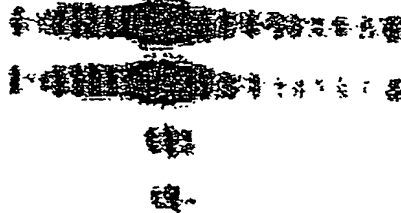
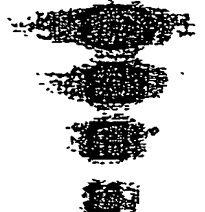
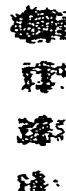
	A	B	C	D
Extra ssDNA	-	+		
Eco SSB	-	-	+	+
time at 37°C	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'	10' 20' 30' 40'
				

FIG. 7

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Effect of *Eco* SSB on the state of template DNA
and product RNA in vRNAP transcription

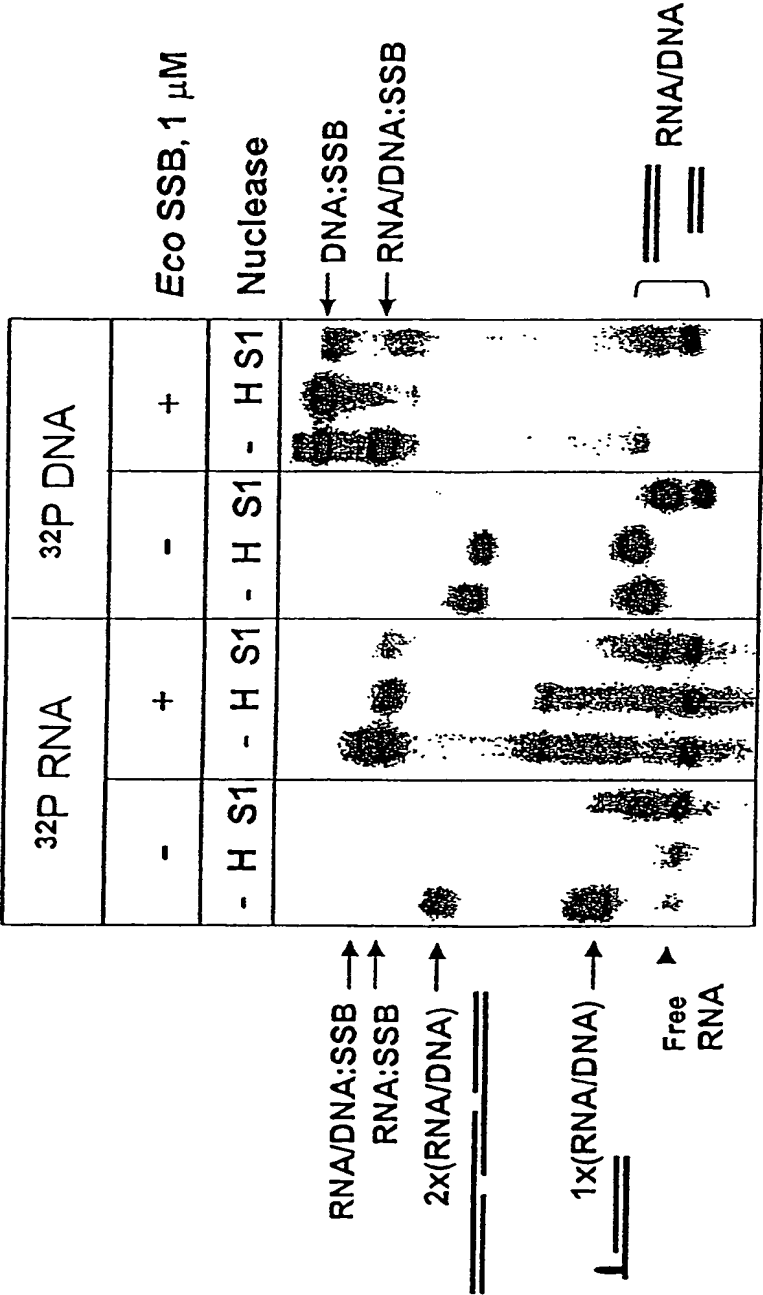


FIG. 8

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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Transcription initiation by vRNAP and mini-vRNAP

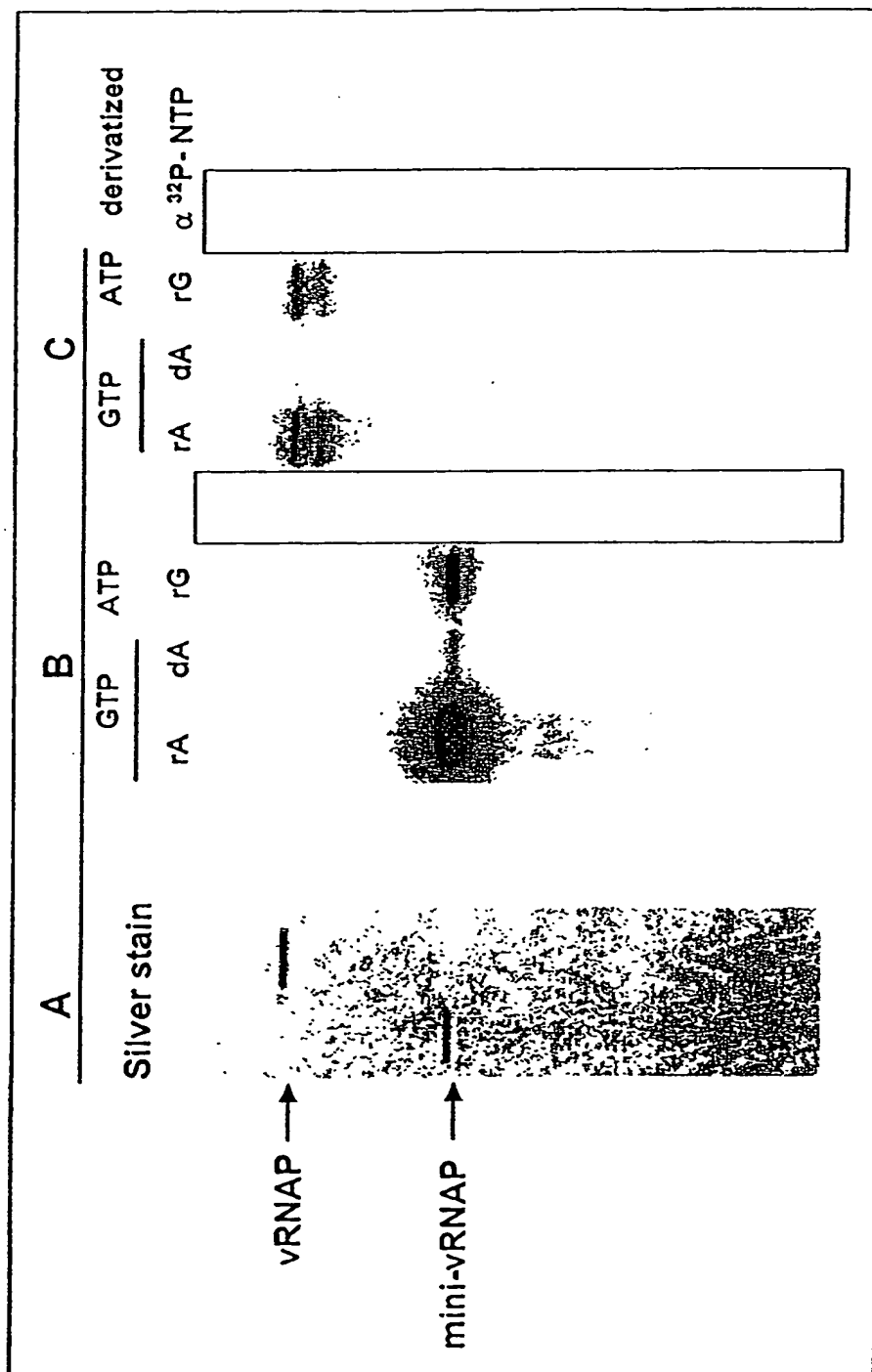


FIG. 9

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Effect of *Eco* SSB on transcription of vRNAP and mini-RNAP

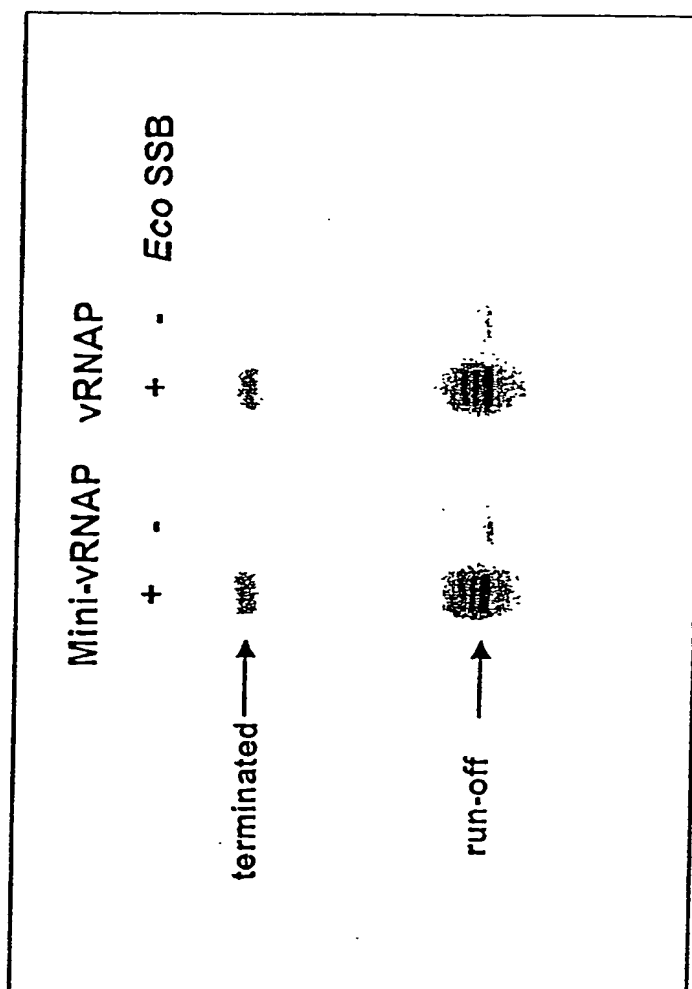


FIG. 10

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION SUBSTRATES FOR SYNTHESIS OF TRANSCRIPTION PRODUCTS CORRESPONDING TO TARGET SEQUENCES

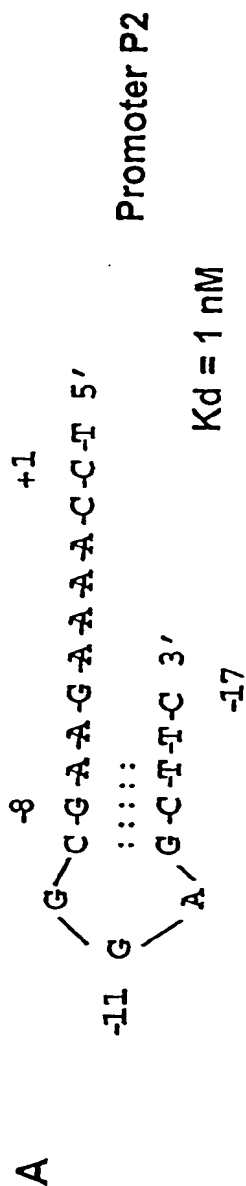
Inventor(s): Gary A. Dahl/Jerome J. Jendrisak/Elena K. Davydova/
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Determination of mini-vRNAP promoter contacts



B

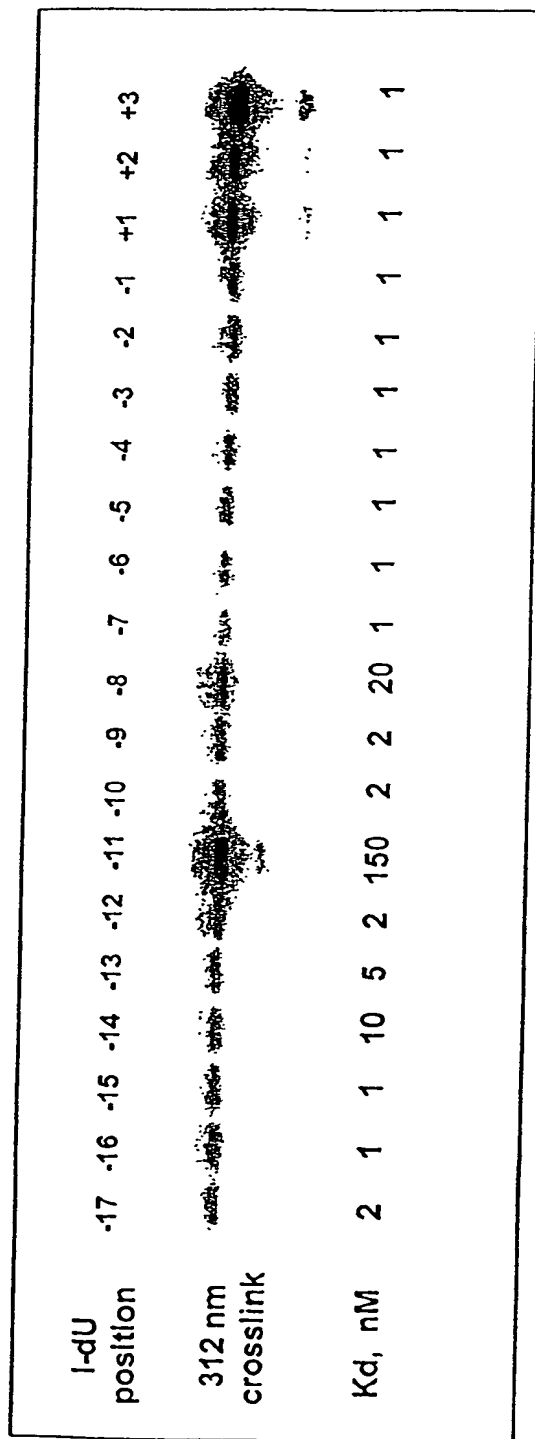
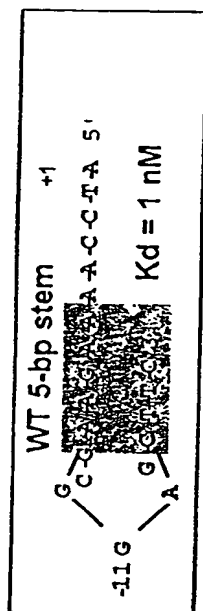


FIG. 11

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Binding affinities of stem-length promoter mutants



Long-stem mutants

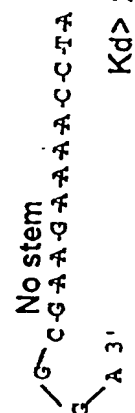
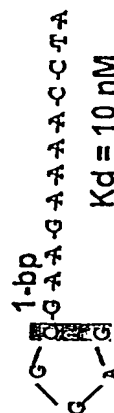
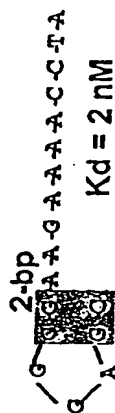
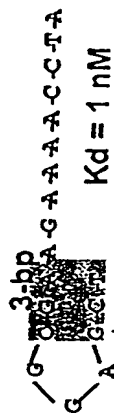


FIG. 12

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Identification of the transcription start site by catalytic
autolabeling

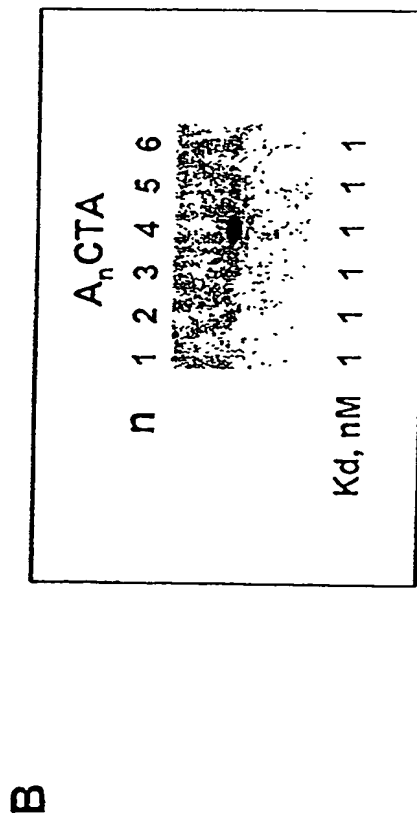


FIG. 13

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UV crosslinking of mutant mini-vRNAPases
to promoter oligonucleotides

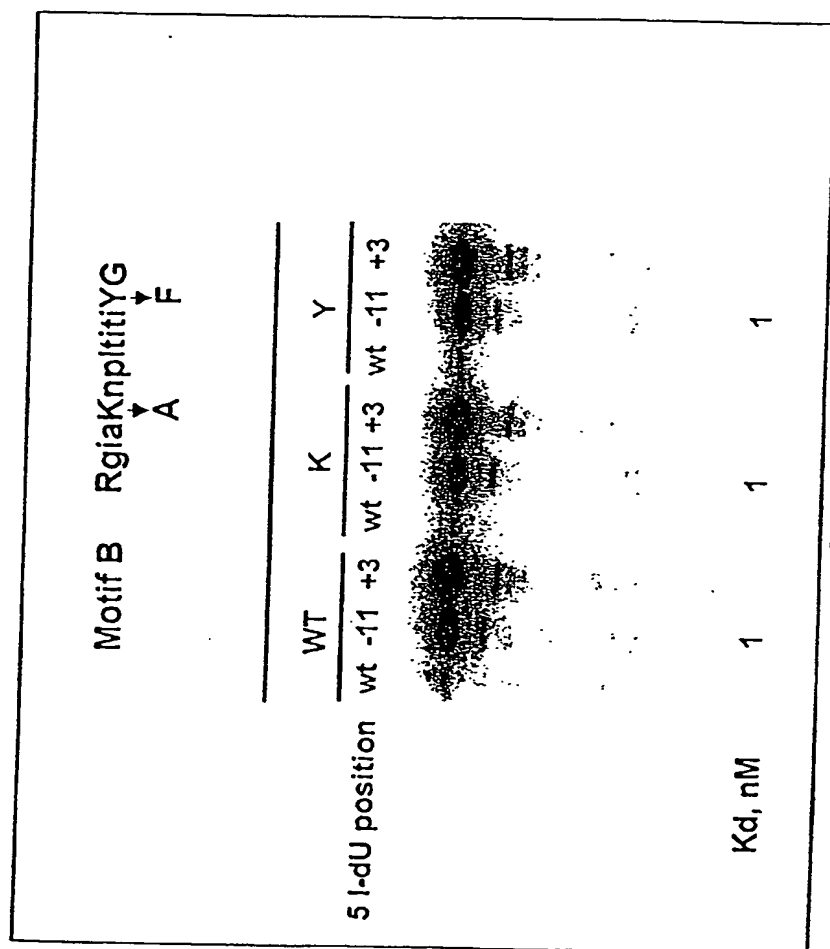


FIG. 14

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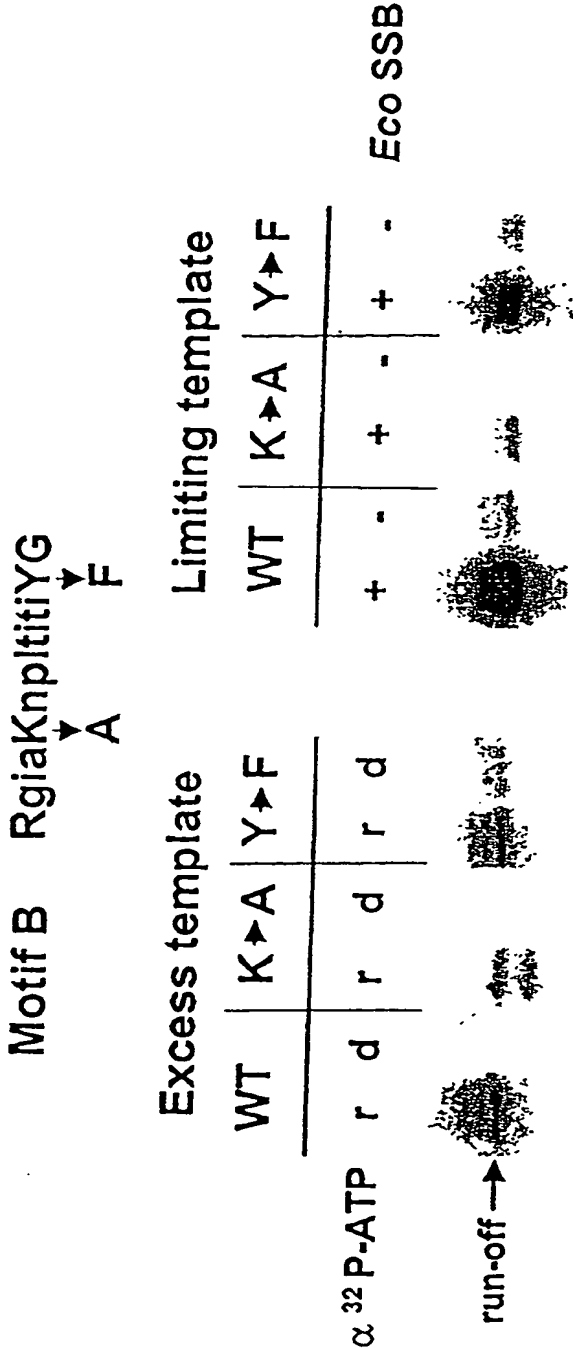
Inventor(s): Gary A. Dahl/Jerome J. Jendrisak/Elena K. Davydova/
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Run-off transcription by mutant mini-vRNAPases



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Detection of *in vivo* activities of N4 vRNAP and mini-vRNAP

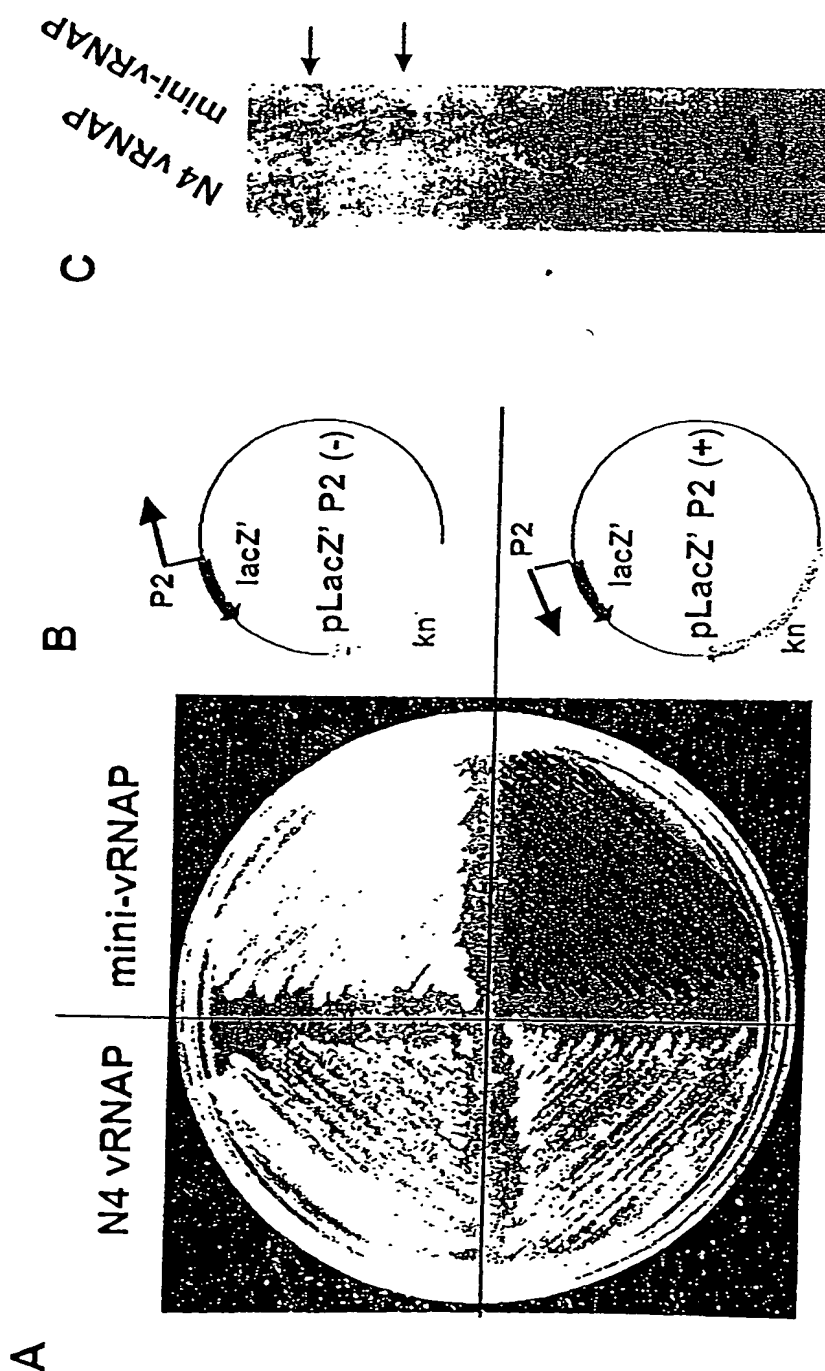


FIG. 17

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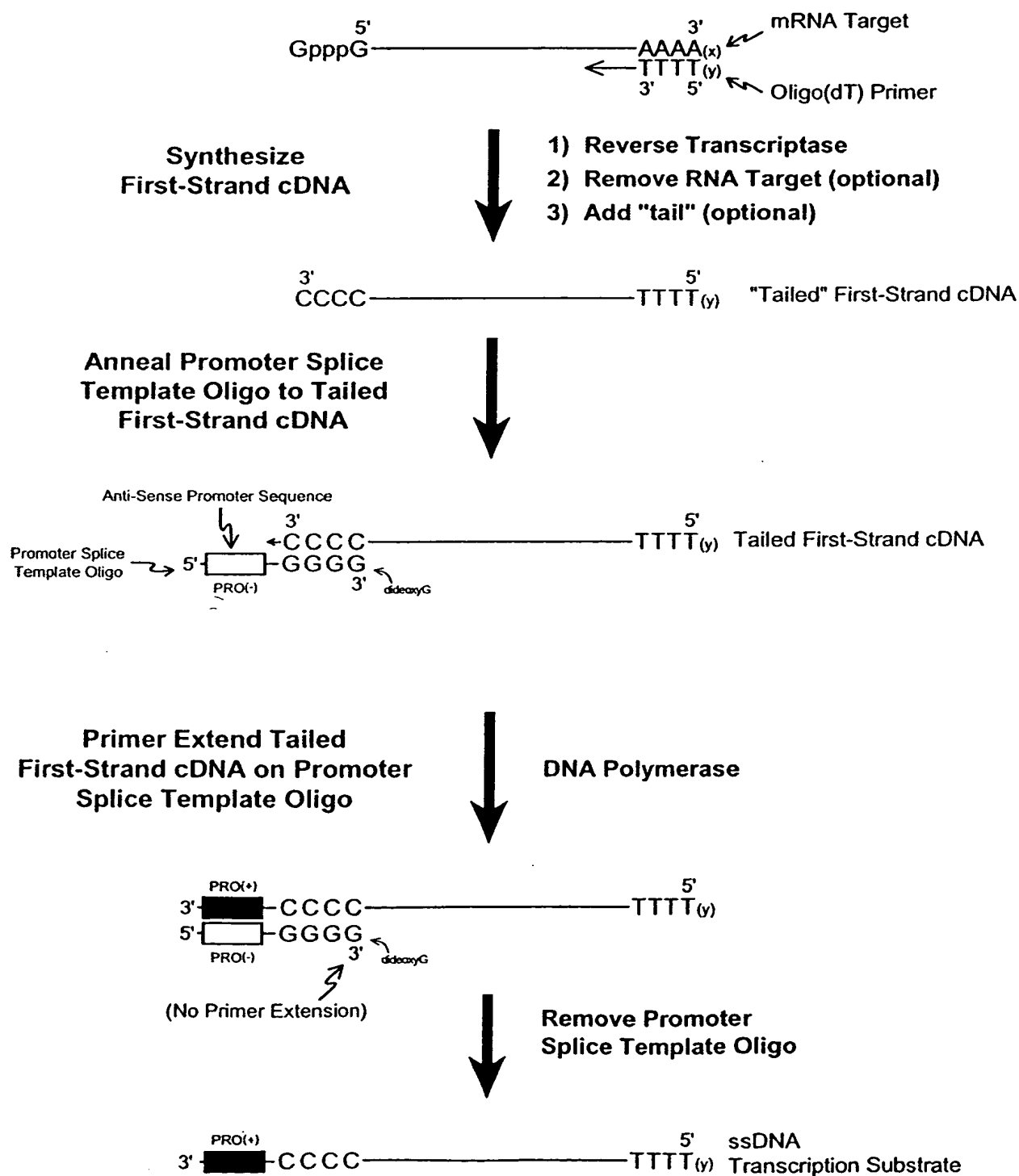


FIG 18

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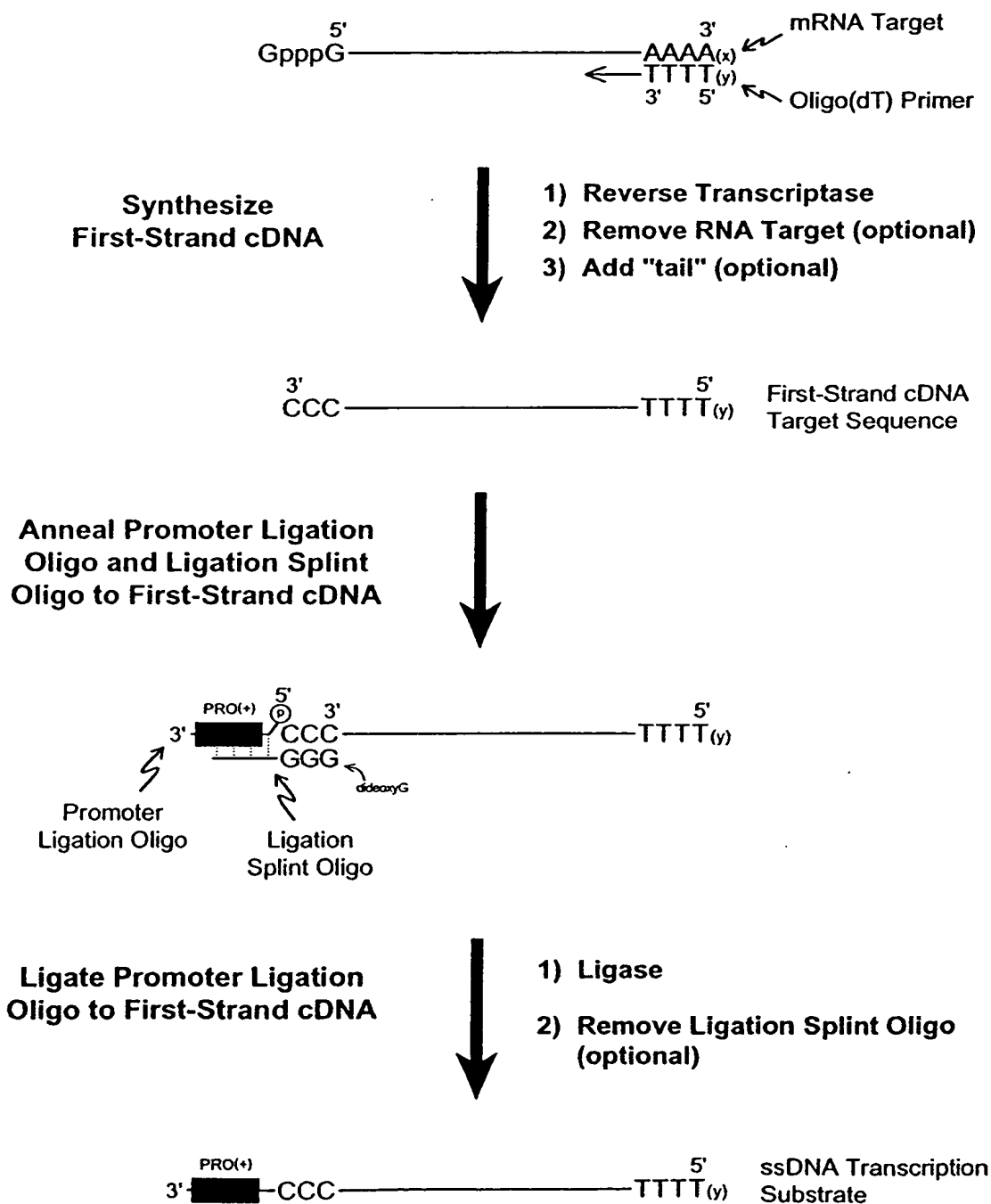


FIG 19

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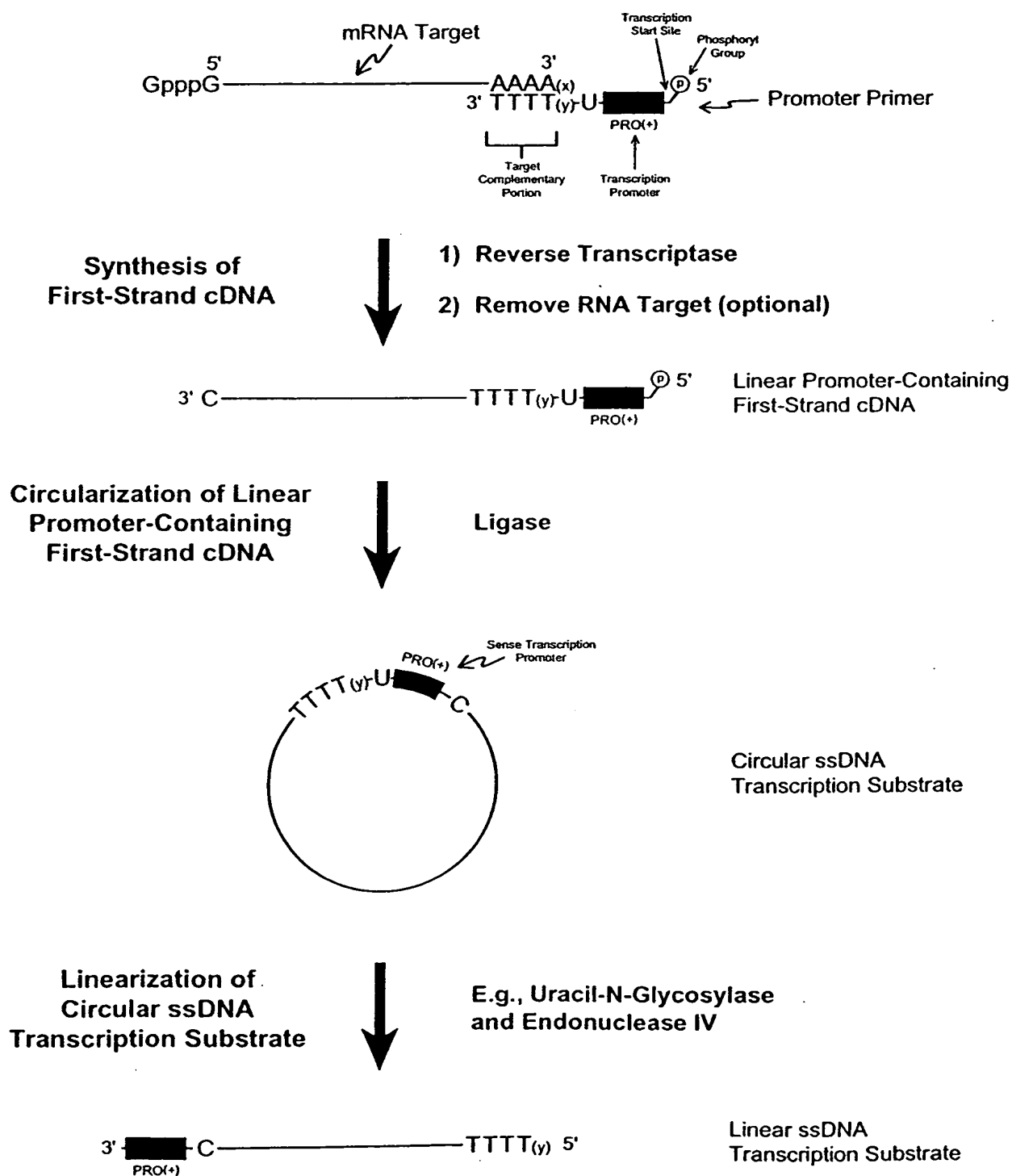


FIG 20

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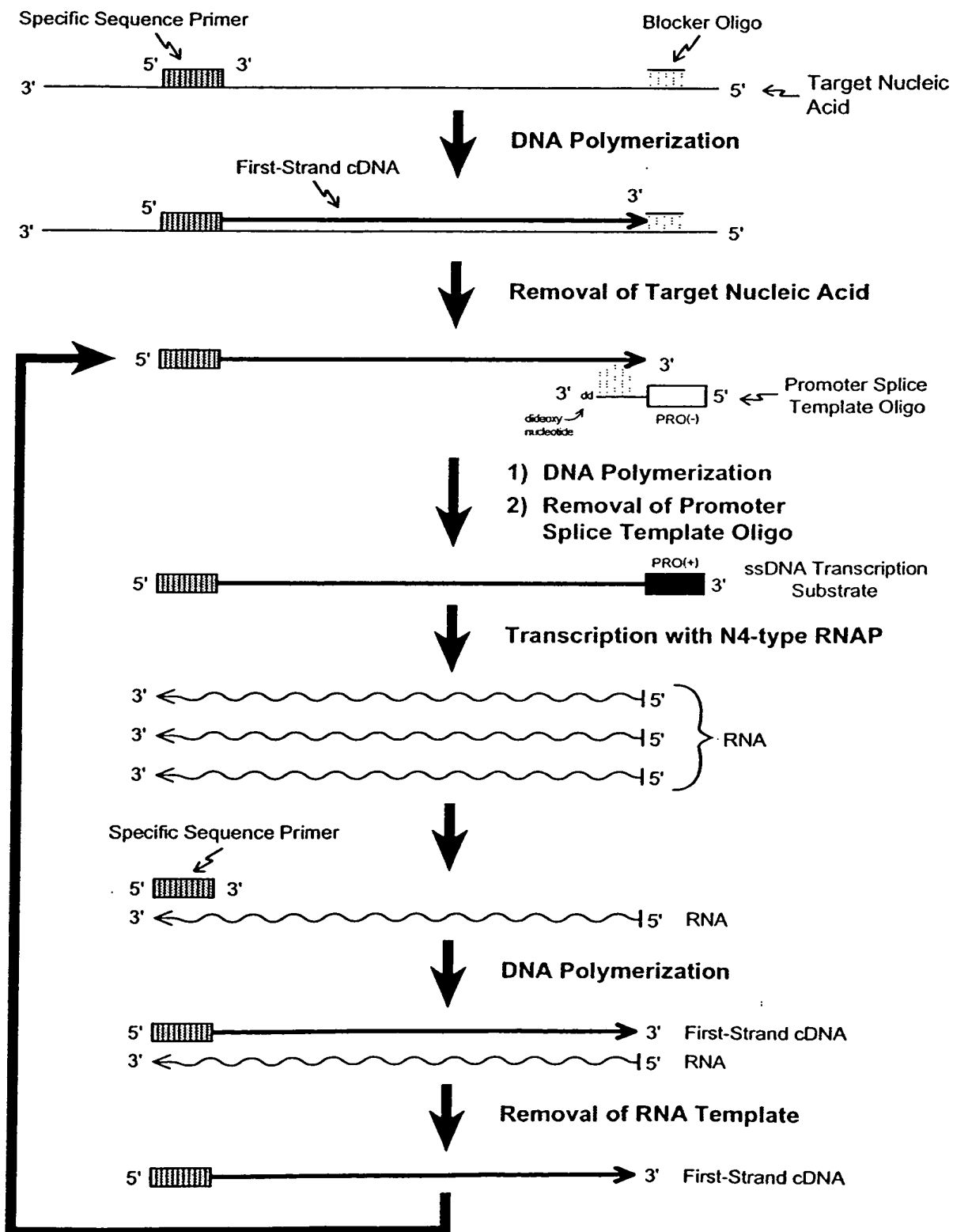


FIG 21

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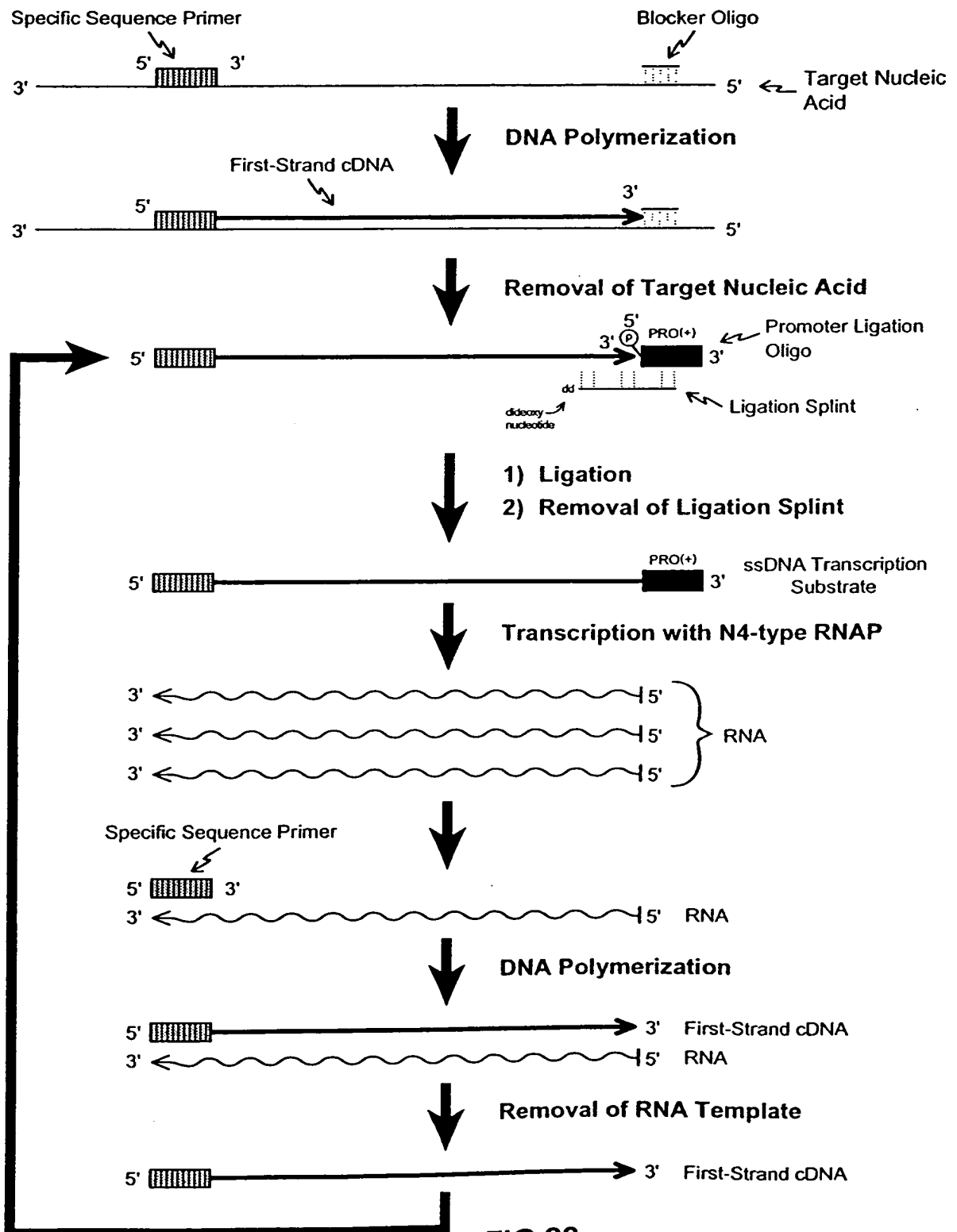


FIG 22

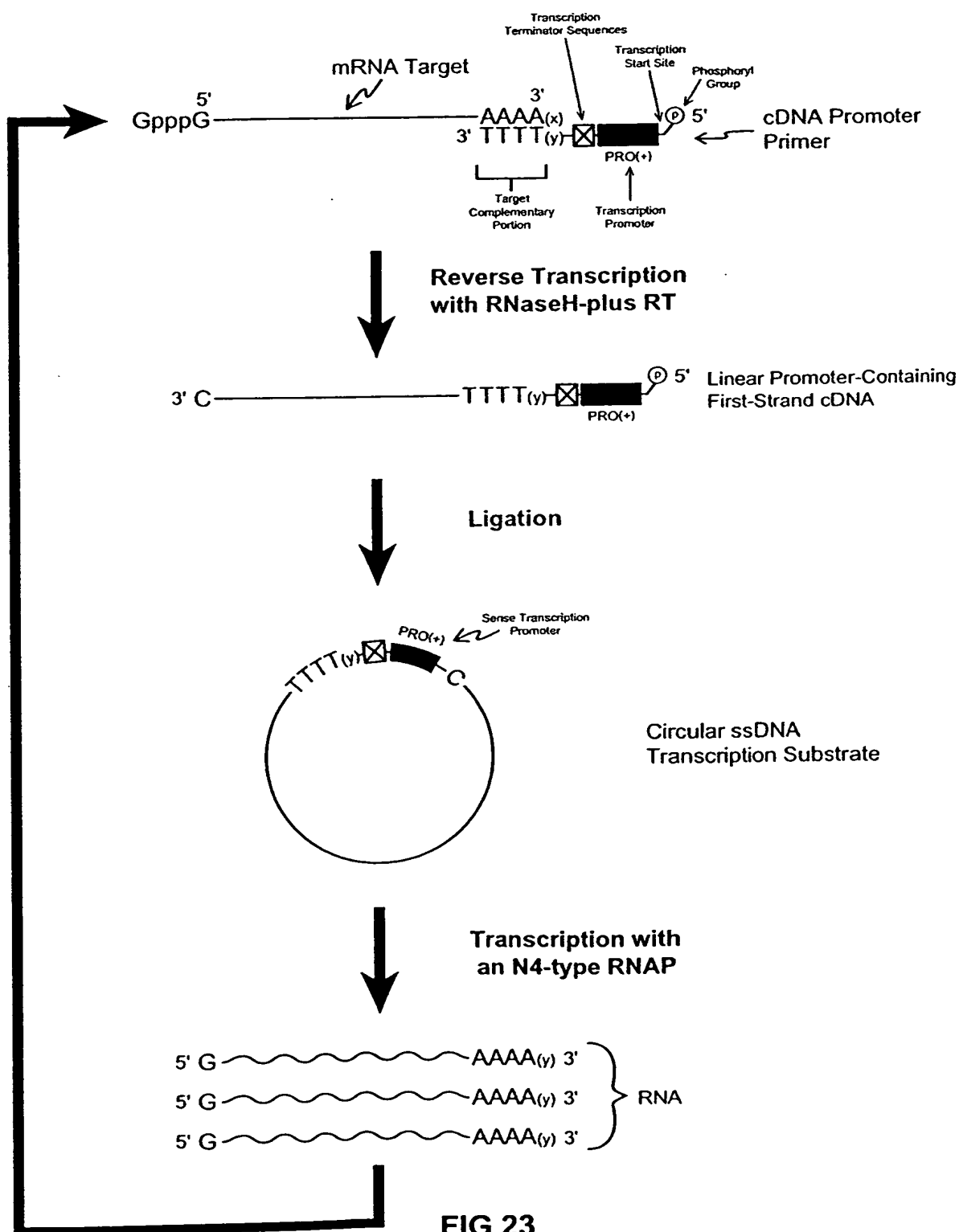
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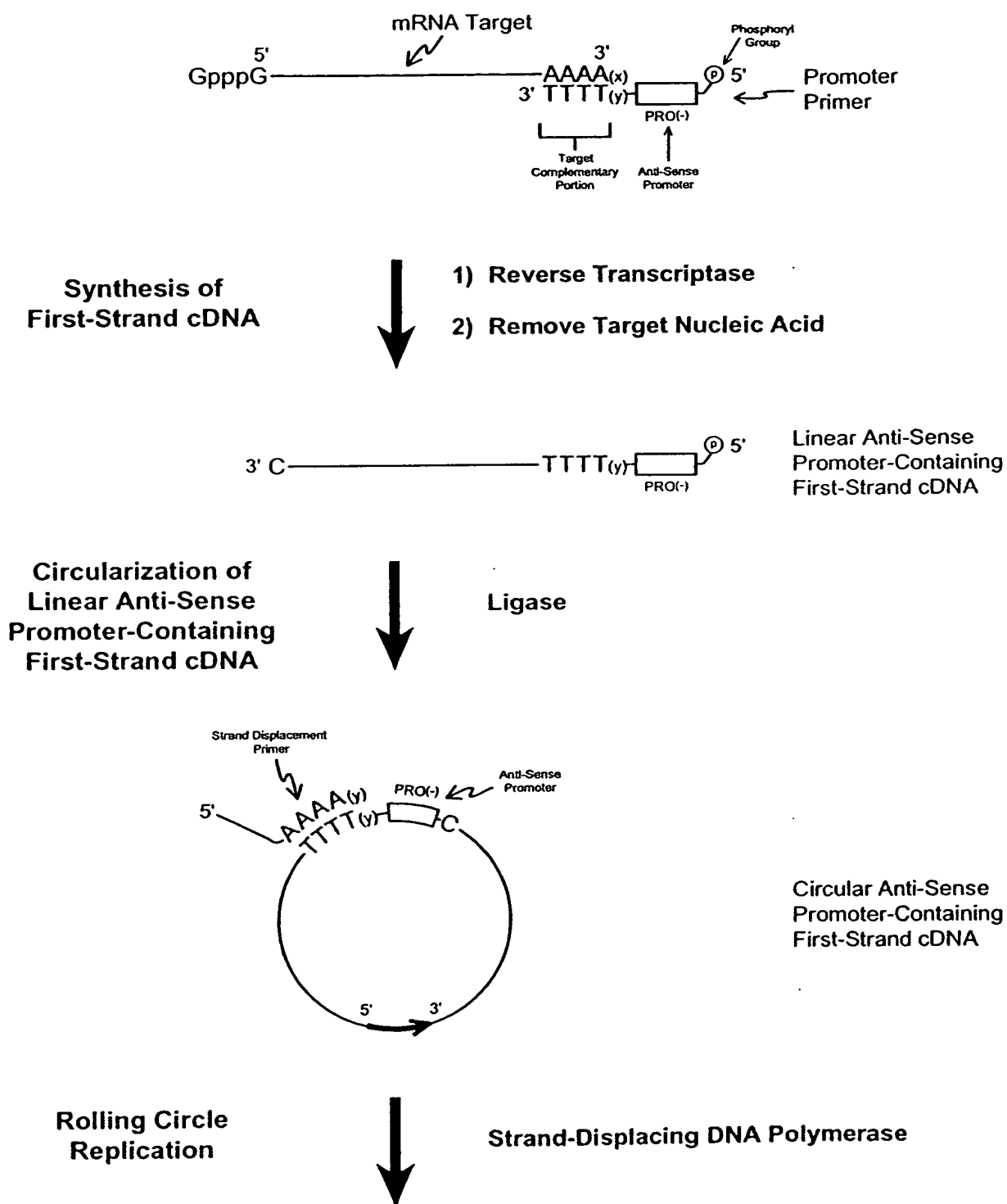


FIG 24 (continued on next page)

PREPARATION AND USE OF SINGLE-STRANDED TRANSCRIPTION
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